UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

CUMENTATION PAGE

AD-A200 203

DTIC FILE COPY

(2)

1a. REPORT

1b. RESTRICTIVE MARKINGS

2a. SECURITY

2b. DECLASSIF

SCHEDULE

AUG 18 1988

S D

3. DISTRIBUTION/AVAILABILITY OF REPORT

Approved for public release; distribution unlimited.

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

Technical Report #10

5. MONITORING ORGANIZATION REPORT NUMBER(S)

AFOSR-TR- 88-0774

6a. NAME OF PERFORMING ORGANIZATION

Texas A&M University

6b. OFFICE SYMBOL (If applicable)

7a. NAME OF MONITORING ORGANIZATION

Air Force Office of Scientific Research

6c. ADDRESS (City, State and ZIP Code)

College Station, TX 77853

7b. ADDRESS (City, State and ZIP Code)

Bolling AFB, DC 20332

8a. NAME OF FUNDING/SPONSORING ORGANIZATION

AFOSR

8b. OFFICE SYMBOL (If applicable)

NM

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

AFOSR-84-0149620-85-C-0144

8c. ADDRESS (City, State and ZIP Code)

Bolling Air Force Base
Washington, DC 20332

10. SOURCE OF FUNDING NOS.

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT NO.
61102F	2304	A6	

11. TITLE (Include Security Classification)

VARIANCE FUNCTIONS AND THE MINIMUM DETECTABLE CONCENTRATION IN ASSAYS

12. PERSONAL AUTHOR(S)

Davidian, M., Carroll, R.J. and Smith, W.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Yr., Mo., Day)	15. PAGE COUNT
Technical Journal	FROM 8/87 TO 8/88		23

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB. GR.	
			calibration, generalized least squares, heteroscedasticity prediction, weighted least squares.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Assay data are often fitted by a nonlinear regression model incorporating heterogeneity of variance. Typically, the standard deviation of the response is taken to be proportional to a power θ of the mean. There is considerable empirical evidence suggesting that for assays of a reasonable size, how one estimates the parameter θ does not greatly affect how well one estimates the mean regression function. An additional component of assay analysis is the estimation of auxillary constructs such as the minimum detectable concentration, for which many definitions exist; we focus on one such definition. The minimum detectable concentration depends both on θ and the mean regression function. We compare standard methods of estimating the parameter θ due to Rodbard (1978), Raab (1981a) Sadler & Smith (1985) and Carroll and Ruppert (1982b). When duplicate counts are taken at each concentration, the first method is only 20% efficient asymptotically in comparison to the fourth for normal data, and in an example the resulting estimate of the minimum detectable concentration is asymptotically 3.7 times more variable. Less dramatic results

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT

UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS ☐

21. ABSTRACT SECURITY CLASSIFICATION

UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE NUMBER (Include Area Code)	22c. OFFICE SYMBOL
Major Brian Woodruff	(202) 767-5026	NM

DD FORM 1473, 83 APR EDITION OF 1 JAN 73 IS OBSOLETE.

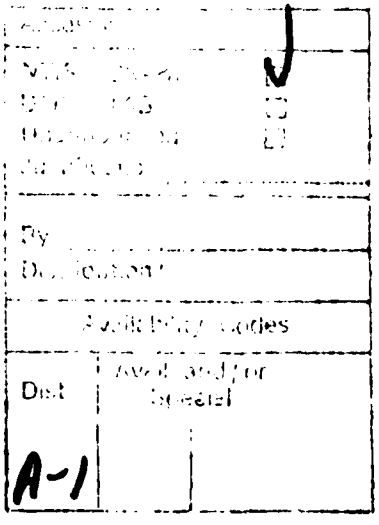
UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

obtain for the second and third estimators compared to the fourth. Simulation results and an example support the asymptotic theory. The results have implications in applications other than the assay problem in which heterogeneity of variance and issues of calibration arise.

VARIANCE FUNCTIONS AND THE MINIMUM DETECTABLE CONCENTRATION IN ASSAYS

Technical Report #10

M. Davidian, R.J. Carroll and W. Smith



VARIANCE FUNCTIONS AND THE

MINIMUM DETECTABLE CONCENTRATION IN ASSAYS

M. Davidian

Department of Statistics
North Carolina State University
Campus Box 8203
Raleigh, North Carolina, U.S.A.
27695-8203

R.J. Carroll

Department of Statistics
Texas A & M University
College Station, Texas, U.S.A.
77843

W. Smith

Statistical and Mathematical Services
Eli Lilly & Company
307 East McCarty Street
Indianapolis, Indiana, U.S.A.
46285

SUMMARY

Assay data are often fitted by a nonlinear regression model incorporating heterogeneity of variance. Typically, the standard deviation of the response is taken to be proportional to a power θ of the mean. There is considerable empirical evidence suggesting that for assays of a reasonable size, how one estimates the parameter θ does not greatly affect how well one estimates the mean regression function. An additional component of assay analysis is the estimation of auxillary constructs such as the minimum detectable concentration, for which many definitions exist; we focus on one such definition. The minimum detectable concentration depends both on θ and the mean regression function. We compare standard methods of estimating the parameter θ due to Rodbard (1978), Raab (1981a) Sadler & Smith (1985) and Carroll and Ruppert (1982b). When duplicate counts are taken at each concentration, the first method is only 20% efficient asymptotically in comparison to the fourth for normal data, and in an example the resulting estimate of the minimum detectable concentration is asymptotically 3.7 times more variable. Less dramatic results obtain for the second and third estimators compared to the fourth. Simulation results and an example support the asymptotic theory. The results have implications in applications other than the assay problem in which heterogeneity of variance and issues of calibration arise.

Some key words: Calibration; Generalized Least Squares; Heteroscedasticity; Prediction; Weighted Least Squares.

1. INTRODUCTION

Recent work in the analysis of assay data in the clinical and biological sciences suggests that these data can be markedly heteroscedastic. In radioimmunoassay, this characteristic has been observed repeatedly and incorporated into the analysis as discussed by Finney (1976), Rodbard (1978), Tiede & Pagano (1979), Raab (1981a,b), Butt (1984) and Sadler & Smith (1985). Such analyses are for the most part special cases of the heteroscedastic nonlinear regression model. Specifically, we observe independent counts Y_{ij} at concentrations x_i for $i = 1,\ldots,N$ and $j = 1,\ldots,M_i$ with means and variances given by

$$E(Y_{ij}) = \mu_i = f(x_i,\beta) \quad ; \quad var(Y_{ij}) = \{\sigma g(x_i,\beta,\theta)\}^2, \tag{1.1}$$

where β is the unknown regression parameter vector of length p, g is the variance function, and θ is the structural variance parameter. A standard model for the mean in a radioimmunoassay is the four parameter log-logistic model

$$f(x,\beta) = \beta_1 + (\beta_2 - \beta_1)/[1 + \exp\{\beta_4(\log x - \beta_3)\}]. \tag{1.2}$$

Almost without exception, the variances have been modeled as functions of the mean response, usually either as a quadratic or as a power of the mean, e.g.,

$$\sigma_i = \text{Standard deviation of } Y_{ij} = \sigma g(x_i,\beta,\theta) = \sigma f(x_i,\beta)^{\theta}. \tag{1.3}$$

The fundamental contribution of Rodbard and other workers has been to

incorporate the heterogeneity into the analysis, thus improving the quality of statistical analysis.

Fitting model (1.1) in assays is discussed by many authors, see Rodbard & Frazier (1975), Raab (1981a), and Finney (1976). The most common method of estimating β is generalized least squares, in which one forms estimates of the σ_i and then estimates β by weighted least squares. For estimating the weights, both formal estimation procedures for estimating θ (Rodbard & Frazier, 1975 and Raab, 1981a) and the method of setting θ to a predetermined fixed value based on experience with a particular assay (Finney, 1976) have been advocated. We consider estimation of the variance parameter to be an important problem independently of whether or not θ is set to a fixed value in curve fitting and subsequent analyses. For example, estimates of θ from developmental and validation runs of an assay are commonly used to establish a fixed value, or range of acceptable values, for θ, as in the example of Section 5. Also, routine calculation of θ after assay implementation provides information that can be helpful in monitoring for assay changes. In the development, production, and quality control of a pharmaceutical product, for example, assays characteristically include fewer test samples and greater numbers of known concentrations than in many clinical applications. This is particularly true when the substance of interest is at very low levels in the final product. In this case, it is practical to estimate θ, as well as limits of assay reliability as discussed below. Extreme deviations from historical values of these parameters may be considered when evaluating the acceptability of an assay. The intention of this paper is to demonstrate that how well one estimates the variance function can be crucial in determining the properties of analyses based on the fitted curve. Qualitatively, then, how θ is characterized, whether by formal estimation or as fixed, is an important issue.

When θ is formally estimated, as discussed by Jobson & Fuller (1980) and Carroll & Ruppert (1982b), $\hat{\beta}$ has the same asymptotic normal distribution as the weighted least squares estimator with known σ_i. The first order asymptotics can be optimistic, but in our experience for RIA and ELISA assays, the asymptotics are often reasonable. Thus, if our only interest is to estimate β, in many assays the method of estimating the variance function may not be crucial. However, the assay problem does not always stop with estimating β, but also addresses issues of calibration, such as confidence intervals for a true x_* given a new Y_*, the classic calibration problem, and determining the sensitivity of the assay using such concepts as the minimum detectable concentration of Rodbard (1978) and the critical level, detection level and determination limit of Oppenheimer, et al. (1983). With the increase in laboratory automation and the use of computers, routine estimation of assay detection limits is becoming commonplace. As noted in practice by Oppenheimer, et al. (1983) and others, a feature of these calibration problems is that efficiency of estimation is essentially determined by how well one estimates θ. This paper provides theoretical and empirical evidence to justify this claim. The qualitative implications of this result are of interest regardless of how estimation of the variance structure is performed. For example, Finney (1978, p. 342) describes how different assumptions about the value of θ can markedly affect the length of confidence intervals in potency estimation for RIA assays.

To the best of our knowledge, our paper is one of the first which shows explicitly that how one estimates the structural variance parameter θ can be important in determining the behavior of estimates of interesting quantities; this conclusion extends beyond the realm of the assay problem. Far from being only a nuisance parameter, θ has an important role in the analysis of calibration and prediction problems. The implications are of interest when θ

is important in itself, as in, for example, off line quality control, see Box (1987) who discusses finding the levels of x which give minimum variance subject to a constraint on the mean.

For our development, instead of pursuing a fully general theory, we focus on the determination of minimum detectable concentration. There is no unique definition of this concept, and for illustration we pick one of the possible candidates (Rodbard, 1978).

Definition. Let $\bar{Y}(x,M)$ be the mean response based on M replicates at concentration level x, taken independently of the calibration data set $\{Y_{ij}\}$ and let $f(0,\beta)$ be the expected response at zero concentration. The minimum detectable concentration x_c at level $(1-\alpha)$ is the smallest concentration x for which

$$\text{pr}\{\bar{Y}(x,M) \geq f(0,\beta)\} > 1 - \alpha. \tag{1.4}$$

Other definitions replace β with $\hat{\beta}$ in (1.4). If $t(\alpha,N-p)$ is the $(1-\alpha)^{th}$ percentile of the t-distribution with N-p degrees of freedom, the usual estimate $\hat{x}_c$ of x_c satisfies

$$\{f(\hat{x}_c,\hat{\beta}) - f(0,\hat{\beta})\}^2 = \{t(\alpha,N-p)\}^2\{\hat{\sigma}^2 g^2(\hat{x}_c,\hat{\beta},\hat{\theta})/M + v[f(0,\hat{\beta})]\}, \tag{1.5}$$

where $v[f(0,\hat{\beta})]$ is an estimate of the variance of $f(0,\hat{\beta})$ and $\hat{\sigma}^2$ is the usual mean squared error from the weighted fit. To be precise one would replace the t-percentage point with a correction based on the limit distribution of the estimates of (β,θ,σ), but this has not been followed in practice since the limit distribution has been unknown and the effect is asymptotically

unimportant. A heuristic discussion of how estimation of the variance structure plays a role in the determination of calibration quantities can be found in Davidian & Carroll (1987).

In Section 2, we discuss three standard methods for estimating the variance parameter θ. In Section 3 we show that, under relatively general conditions, two of these can be quite a bit less efficient than the third and discuss how this difference translates theoretically to the minimum detectable concentration problem. In assays, along with standard observations at known concentrations, there are also test samples composed of additional observations at unknown concentrations. The first two estimators can incorporate information from both standards and unknowns while the third is equipped only to use information from standards. We discuss how to combine estimators to accomodate both types of data with an increase in efficiency. In Sections 4 and 5, we discuss a small Monte-Carlo study and an example to illustrate the results. The key conclusion is that how one estimates θ can affect the relative efficiency of estimated quantities useful in the calibration of assays.

2. METHODS OF ESTIMATING MEAN AND VARIANCE PARAMTETERS

The problem of estimating θ in models (1.1) and (1.3) has been discussed in many places in the literature. See, for example, Rodbard (1978), Jobson & Fuller (1980), Raab (1981a), Carroll & Ruppert (1982b) and for a general theory Davidian & Carroll (1987). We focus our attention on three methods, two of which were proposed in the assay context. For simplicity, we discuss only the case of equal replication $M_i = M \geq 2$. The first two methods require some replication and do not use the form of the mean response so that standards and unknowns both may be considered.

2.1 Log-linearized estimation

Model (1.3) implies upon taking logarithms that the log standard deviation is linear in the log mean with slope θ. If $(\bar{Y}_i, S_i^2)$ are the within concentration sample means and variances, this suggests estimation of θ as the slope from regressing log S_i on log $\bar{Y}_i$. If we denote this estimate as $\hat{\theta}_{LL}$, Rodbard (1978) suggests forming estimated standard deviations as the sample means to the power $\hat{\theta}_{LL}$, and then applying weighted least squares to estimate β.

2.2 Modified maximum likelihood

Assuming independence, Raab (1981a) suggests estimating θ without making any assumptions about the form of the mean function. Raab proposes estimation of θ by joint maximization of the "modified" normal likelihood

$$\prod_{i=1}^{N} \{2\pi\sigma^2 g(\mu_i,\theta)\}^{(M-1)/2} \exp\left[-\sum_{j=1}^{M} (Y_{ij}-\mu_i)^2/(2\sigma^2 g^2(\mu_i,\theta))\right] \qquad (2.1)$$

in the parameters σ, θ, $\mu_1,\ldots,\mu_N$, where we have written $g(x_i,\beta,\theta)$ as $g(\mu_i,\theta)$ to emphasize the dependence of the variance function on the mean response. Estimation of β may now proceed via weighted least squares in a fashion analogous to the log-linearized method. Sadler & Smith (1985) maximize (2.1) in σ and θ but with μ_i estimated by $\bar{Y}_i$. Their estimate of θ is easier to compute and asymptotically equivalent to that of Raab under the conditions of Section 3.

2.3 Pseudo-likelihood

For given $\hat{\beta}$ the pseudo-likelihood estimator of θ is the normal theory maximum likelihood estimate, maximizing

$$-M\sum_{i=1}^{N} \log\{g(x_i,\hat{\beta},\theta)\} - (N/2)\log\left[N^{-1}\sum_{i=1}^{N}\sum_{j=1}^{M} \{Y_{ij}-f(x_i,\hat{\beta})\}^2 g(x_i,\hat{\beta},\theta)^{-2}\right].$$

see Carroll & Ruppert (1982b). To estimate θ and β jointly one can (i) set $\hat{\beta}$ = unweighted least squares; (ii) estimate θ by pseudo-likelihood; (iii) form estimated variances $g^2(x_i,\hat{\beta},\hat{\theta})$; (iv) re-estimate β by weighted least squares; (v) iterate (ii) - (iv) one or more times. The number of cycles $\mathcal{C}$ of this algorithm is the number of times one performs step (iv). One can do step (ii) by direct maximization or by weighted least squares as in Davidian & Carroll (1987).

Pseudo-likelihood requires no replication and easily copes with unequal replication. Since pseudo-likelihood depends on the form of the mean function, only data from standards may be used to construct the estimate.

2.4 Other methods

Other methods have been proposed; see Jobson & Fuller (1980) and Box & Hill (1974). Robust variance function estimation methods have also been developed by Carroll & Ruppert (1982b) and Giltinan, Carroll & Ruppert (1986).

A final method of jointly estimating (β,θ) is normal theory maximum likelihood. There are important issues of robustness which complicate routine use of this method, see McCullagh (1983), Carroll & Ruppert (1982a) and Davidian & Carroll (1987) for further discussion. For assay data, pseudo-likelihood and maximum likelihood estimates of θ have similar asymptotic behavior, and we use the former largely for its ease of calculation.

At this point we address briefly issues related to assay design. It may be argued that estimation of the variance structure ignores the difficulties introduced by lack of randomization, in particular that adjacent positioning of replicates will likely lead to underestimation of the variance. This is an important but difficult issue which involves problems of variance component estimation and correlated observations, and we consider it beyond the scope of

the discussion here. It is worthwhile to note that the pseudo-likelihood estimator includes lack-of-fit error as well as replication error while the log-linearized estimator does not include the former. The two methods do not estimate the same quantity without the proper assumptions.

3. ASYMPTOTIC THEORY

The asymptotic theory of the log-linearized estimator, $\hat{\theta}_{LL}$, is complicated because regressing $\log S_i$ on $\log \bar{Y}_i$ is not a standard linear regression problem. Likewise, the asymptotic theory for the modified maximum likelihood estimator $\hat{\theta}_{MML}$ is complicated because the dimension of the parameter space increases with N. Both problems may be treated as nonlinear errors-in-variables problems as in Wolter & Fuller (1982) and Stefanski & Carroll (1985). The error in estimating μ_i by $\bar{Y}_i$ in $\hat{\theta}_{LL}$ or by the joint estimator $\hat{\mu}_i$ in $\hat{\theta}_{MML}$ causes these estimators to be biased asymptotically. This bias is typically negligible, because in most assays the parameter σ in (1.1) is quite small. It thus makes sense to define an asymptotic theory where the sample size $N_S = NM$ becomes large and σ simultaneously is small. Because in most assays the number of replicates M is small, we let $N \to \infty$ and $\sigma \to 0$ while keeping M fixed; Raab (1981a) suggests that $M = 2$ is most common. The approach of letting $N \to \infty$ and $\sigma \to 0$ simultaneously is dictated by the problems of studying $\hat{\theta}_{LL}$ and $\hat{\theta}_{MML}$. The pseudo-likelihood estimator $\hat{\theta}_{PL}$ has routine asymptotic theory even for fixed σ.

The asymptotic distributions of these estimators for θ can be obtained from the general theory of Davidian & Carroll (1987). Throughout this discussion we focus on model (1.3). Define $\epsilon_{ij} = \{Y_{ij} - f(x_i,\beta)\}/\{\sigma f(x_i,\beta)^{\theta}\}$, $v_i = \log f(x_i,\beta)$, $q_i^2 = (M-1)^{-1}\Sigma(\epsilon_{ij}-\bar{\epsilon}_i)^2$, and $\sigma_v^2 = \lim (N-1)^{-1}\Sigma(v_i-\bar{v})^2$.

THEOREM 1. As $N \to \infty$ and $\sigma \to 0$ simultaneously and $N^{1/2}\sigma = O(1)$, if the random variables $\{\epsilon_{ij}\}$ are symmetric and independent and identically distributed, then $\hat{\theta}_{LL}$, $\hat{\theta}_{MML}$ and $\hat{\theta}_{PL}$ are asymptotically normally distributed with mean θ and variances $\text{var}\{\log q_i\}/(4N\sigma_v^2)$, $\text{var}(q_i^2)/(4N\sigma_v^2)$, and $\text{var}(\epsilon_{ij}^2)/(4NM\sigma_v^2)$, respectively.

Here, the symmetry condition is necessary to ensure that the asymptotic distributions of $\hat{\theta}_{LL}$ and $\hat{\theta}_{MML}$ have zero mean; symmetry is unnecessary for the result for $\hat{\theta}_{PL}$. Since $\text{var}(q_i) = \{\text{var}(\epsilon_{ij}^2)/M\} + [2/\{M(M-1)\}]$, $\hat{\theta}_{MML}$ has uniformly larger asymptotic variance than $\hat{\theta}_{PL}$ for all $M \geq 2$ regardless of the distribution of the $\{\epsilon_{ij}\}$. For normal data, the efficiency of modified maximum likelihood relative to pseudo-likelihood is $(M-1)/M$. A simple calculation shows in this case that the efficiencies of the log-linearized method relative to modified maximum likelihood are 40.5%, 60.8%, 71.3%, and 89.3% for M = 2, 3, 4, and 10, respectively, so that the efficiency of $\hat{\theta}_{LL}$ relative to $\hat{\theta}_{PL}$ is only 20.3% for M = 2 and 53.5% for M = 4. In our experience, these numbers slightly exaggerate the inefficiency of $\hat{\theta}_{LL}$ relative to pseudo-likelihood, especially in assay problems where N is rather small, as in the Monte Carlo study of Section 4. The asymptotic relative efficiencies of $\hat{\theta}_{LL}$ relative to $\hat{\theta}_{MML}$ agree quite well with the efficiencies of 39%, 62% and 77% and 39%, 64% and 74% for M = 2, 3 and 4 reported by Raab (1981a) and Sadler & Smith (1985), respectively, in two Monte-Carlo studies with larger N. Davidian & Carroll (1987) show that under the condition $\sigma \to 0$ and (1.1), pseudo-likelihood and normal theory maximum likelihood are asymptotically equivalent. Thus, while the inefficiency of the log-linearized and modified maximum likelihood methods is not surprising for normal data, Theorem 1 shows the large extent to which the log-linearized method can be inefficient for small M under normality as well as the fact that

modified maximum likelihood is inefficient for all distributions.

A benefit of the log-linearized and modified likelihood methods is that they can incorporate the responses at unknown concentrations to estimate θ while pseudo-likelihood can not. One can improve upon pseudo-likelihood to take into account unknowns by considering a weighted estimator of θ which combines $\hat{\theta}_{PL}$ and one of the others in the obvious way. The increase in efficiency of such an estimator over the others alone will depend on the amount of information available from test samples relative to standard samples. In this discussion we have restricted our investigation to standard observations because the improvement in efficiency by pseudo-likelihood comes from these data.

For the minimum detectable concentration, note that in (1.5) the term $v\{f(0,\hat{\beta})\}$ is of the order $(NM)^{-1}$ and is hence small relative to all the other terms. Of course, for normally distributed data, the solution to (1.5) is the quantity x_c^*, where

$$0 = \{z(\alpha)\}^2\sigma^2 f^{2\theta}(x_c^*,\beta)/M - \{f(x_c^*,\beta) - f(0,\beta)\}^2, \tag{3.1}$$

and $z(\alpha)$ is the $(1 - \alpha)^{th}$ percentile point of the standard normal distribution.

Here is the major result, the technical details for which are given in the appendix. Define $d_0 = \log f(0,\beta) - \lim N^{-1}\Sigma \log f(x_i,\beta)$.

THEOREM 2. Let $\hat{x}_c(LL)$, $\hat{x}_c(MML)$ and $\hat{x}_c(PL)$ denote the estimated minimum detectable concentrations using the log-linearized estimate $\hat{\theta}_{LL}$, the modified maximum likelihood estimate $\hat{\theta}_{MML}$ and the pseudo-likelihood estimate $\hat{\theta}_{PL}$, respectively. Then under regularity conditions and the conditions of Theorem 1, there is a constant A_0 and a sequence b_N for which $\hat{x}_c(LL)$, $\hat{x}_c(MML)$ and

$\hat{x}_c(PL)$ are asymptotically normal with mean x_c^* and variances $V_{LL}c_N$, $V_{MML}c_N$ and $V_{PL}c_N$, respectively, where $c_N = \sigma^2/(Nb_N^2A_0^2)$, $V_{LL} = \text{var}(\epsilon_{ij}^2)+\text{var}(\log q_i^2)d_0^2M/\sigma_v^2)$, $V_{MLL} = \text{var}(\epsilon_{ij}^2)+\text{var}(q_i^2)d_0^2M/\sigma_v^2$, and $V_{PL} = \text{var}(\epsilon_{ij}^2)\{1+d_0^2/\sigma_v^2\}$.

From the above, the ordering in efficiency of estimated minimum detectable concentration is the same as the ordering of estimators of θ. The asymptotic relative efficiency of the modified maximum likelihood estimate of minimum detectable concentration relative to the pseudo-likelihood estimate is $\text{var}(\epsilon_{ij}^2)(\sigma_v^2+d_0)/\{\text{var}(\epsilon_{ij}^2)(\sigma_v^2+d_0^2)+2d_0^2/(M-1)\}$, which is less than 1 for all M regardless of the value of $\text{var}(\epsilon_{ij}^2)$. The asymptotic relative efficiency of the log-linearized estimate of minimum detectable concentration to the pseudo-likelihood estimate is $\text{var}(\epsilon_{ij}^2)(\sigma_v^2+d_0^2)/\{\sigma_v^2\text{var}(\epsilon_{ij}^2)+Md_0^2\text{var}(\log\ q_i^2)\}$. Thus, pseudo-likelihood is favored over modified maximum likelihood for any underlying distribution and is favored over the log-linearized method at least when the data are approximately normal. For distributions other than normal, calculations with other symmetric distributions such as double exponential and various contaminated normal distributions show very few cases where $\hat{\theta}_{LL}$ is more efficient than $\hat{\theta}_{PL}$. The numerical efficiencies depend on the logarithm of the true means through d_0^2 and σ_v^2. For example, in the simulation discussed in the next section, the asymptotic relative efficiency of the log-linearized estimate is 26.6% for M = 2 and 62.1% for M = 4.

The asymptotic theory thus confirms that inefficiencies in estimating the variance parameter θ translate into inefficiencies for estimating the minimum detectable concentration.

4. A SIMULATION

To check the qualitative nature of the asymptotic theory, we ran a small

simulation based on an ELISA assay. The responses Y_{ij} were normally distributed with mean and variance satisfying (1.2), (1.3), where $\beta_1 = 30.5754$, $\beta_2 = 1.9173$, $\beta_3 = 1.7235$, $\beta_4 = 0.9730$, $\theta = 0.7$ and $\sigma = 0.0848$. The 12 concentrations were chosen from the example of Section 5 to represent the number of levels typical for assay data. These were every other concentration beginning with 0.000 in Table 1. For each of 500 data sets for M = 2 and 4, β was estimated by unweighted least squares and by generalized least squares with θ estimated by the log-linearized, pseudo-likelihood, and modified maximum likelihood methods, with weighting as in Section 2. We used the estimate of Sadler & Smith (1985) described in Section 2 in place of modified maximum likelihood and computed the pseudo-likelihood estimate for $\mathcal{C} = 2$ cycles of the algorithm as suggested by the results of Davidian & Carroll (1987). The estimates of θ were constrained to lie in the interval $0 \leq \theta \leq 1.50$.

For M = 2, the Monte Carlo biases and standard errors, in parentheses, for θ were -0.0371 (0.2848) for the log-linearized method, -0.0162 (0.2146) for modified maximum likelihood and -0.0107 (0.1799) for pseudo-likelihood. For M = 4 these were -0.0026 (0.1504), -0.0009 (0.1316) and -0.0012 (0.1247), respectively. Thus, Monte Carlo efficiencies relative to pseudo-likelihood based on Monte Carlo mean squared errors were for M = 2 (M = 4) 39.4% (68.7%) for the log-linearized method and 70.1% (89.8%) for modified maximum likelihood, compared to theoretical values of 20.3% (53.5%) and 50.0% (75.0%), respectively. The asymptotics tend to exaggerate the loss of efficiency; nonetheless, this example indicates that the pseudo-likelihood estimator can be in some circumstances a considerable improvement over the other methods.

For the minimum detectable concentration we chose $\alpha = 0.05$. For all methods, a Monte Carlo measure of (1.4) based on generating means of M new responses at $\hat{x}_c$ for each data set showed that this requirement was satisfied;

rather than 0.95, every case was at least 0.97. Mean values of estimated minimum detectable concentrations multiplied by 100 and their Monte Carlo variances relative to pseudo-likelihood (in parentheses) are reported below:

Monte Carlo Results for Minimum Detectable Concentration

	M = 2	M = 4
Least Squares	14.023 (10.5%)	9.747 (8.8%)
Log-linearized	5.102 (33.2%)	3.033 (74.9%)
Modified Maximum Likelihood	4.685 (52.8%)	3.010 (84.6%)
Pseudo-likelihood	4.313 (100.0%)	2.928 (100.0%)

The relatively poor behavior of unweighted least squares is evident. To quote from Oppenheimer, et al. (1983): "Rather dramatic differences have been observed depending on whether a valid weighted or inappropriate unweighted analysis is used." The mean minimum detectable concentration for the log-linearized method was 18% larger than for pseudo-likelihood method for M = 2 and 4% larger for M = 4. Whether raw numerical difference is of any practical consequence will depend on the context. The method of estimating θ seems to have important consequences for the variability of the estimate of minimum detectable concentration. The asymptotics suggest efficiencies of 26.6% for the log-linearized method and 58.8% for modified maximum likelihood when M = 2 and 62.1% and 81.1%, respectively, when M = 4, so overall the asymptotics exaggerate somewhat the loss of efficiency. We also computed the estimates of minimum detectable concentration when θ was known and equal to 0.7, so that the only difference between the methods was whether the generalized least squares weighting depended on sample means or predicted values. In every instance, the mean estimates of minimum detectable concentration and their variances were very close, suggesting that the method

used to estimate θ was responsible for the differences among the estimates and their variances reported above.

The qualitative implication is that the relative efficiency of the estimated minimum detectable concentration can be affected by the algorithm used to estimate the variance parameter θ.

5. AN EXAMPLE

We illustrate the implications of the theory by an example from the development stage of an ELISA assay for which additional concentrations were used to characterize the mean and variance of the standards, see Table 1. The analysis is for illustrative purposes only and shows that the methods can lead to nontrivially different results even with a larger sample size.

For the full data set and reduced data sets considering all possible combinations of duplicates (except one set for which an $S_i^2 = 0$), we computed assuming (1.2) and (1.3) the pseudo-likelihood, log-linearized and Sadler & Smith estimates of θ and x_c and that of x_c based on unweighted least squares. The results are given in Table 2 and show that the three estimates vary greatly. As a crude measure of this we computed the means and standard deviations of $\hat{\theta}$ and $\hat{x}_c$ for the five data sets obtained from duplicates (ignoring the fact that these are not strictly independent). Below we list "relative efficiencies" based on these crude measures:

"Relative Efficiencies" for Estimators of θ and x_c

	LL to PL	MML to PL	LL to MML
$\hat{\theta}$	22.2%	35.1%	63.2%
$\hat{x}_c$	52.9%	65.9%	80.2%

Qualitatively, the estimators exhibit the behavior predicted by the theory; quantitatively, the values compare favorably with the theory given the crudity

of the comparison. The example shows that there can be wide differences among the various methods for estimation of θ and minimum detectable concentration.

6. DISCUSSION

We have addressed the general issue of estimating calibration quantities in assays which exhibit large amounts of heterogeneity. The wrong model assumption of not weighting at all leads to large decreases in the efficiency of analysis. Even when weighting is used, changes in relative efficiency occur depending on the method of estimating the variances, especially the parameter θ in (1.3). The key point is that while for estimation of β the effect of how one estimates the variance function is only second order, for estimation of other quantities such as minimum detectable concentration, the effect is first order. The implications of the results are relevant in other calibration contexts besides the assay problem.

We have had success using the idea of pseudo-likelihood in Carroll & Ruppert (1982b); this method applies in general heteroscedastic models and is easy to compute, although the reader should be aware that it is not robust against outliers. In the assay problem, the pseudo-likelihood method may be combined with one of the others in the case in which standard as well as unknown observations are present, resulting in an increase in efficiency. The gain in efficiency will depend on the particular data set.

One can also consider data transformation rather than weighting. The transform-both-sides idea in Carroll & Ruppert (1984) applies to the assay problem. Further results and descriptions of other methods of variance function estimation are given by Davidian & Carroll (1987).

ACKNOWLEDGEMENT

The research of Carroll and Davidian was supported by the U.S. Air Force Office of Scientific Research.

APPENDIX. PROOFS OF RESULTS.

The analysis of the minimum detectable concentration is complicated by the behavior of the derivative of $f(x,\beta)$ with respect to β at x=0, especially for the standard model (1.2). Write $f(x,\beta) \doteq h(\eta,\beta)$, where $\eta = \ell(x,\beta)$, $\eta_c^* = \ell(x_c^*,\beta)$ and $\hat{\eta}_c = \ell(\hat{x}_c,\hat{\beta})$. In the model (1.2), $\eta = \ell(x,\beta) = \exp(\beta_4 \log x)$. We assume throughout that $f(0,\beta) > 0$, and that all functions are sufficiently smooth. Assume further that

$$\ell(0,\beta) = 0 \; ; \tag{A.1}$$

$$\partial/\partial\eta \; h(0,\beta) = h_\eta(0,\beta) \neq 0 \; ; \tag{A.2}$$

$$\ell_\beta(0,\beta) = 0 \; ; \tag{A.3}$$

If $w \to 0$ and v is a random variable such that

$$\text{p-lim } \ell(v,\beta)/\ell(w,\beta) = 1, \text{ then}$$

$$\text{p-lim sup}\{ \; | \; \ell_\eta(\alpha v+(1-\alpha)w,\beta)/\ell_\eta(w,\beta) - 1 \; | \text{ for } 0\leq\alpha\leq 1 \; \} = 0 \; . \tag{A.4}$$

These assumptions are satisfied for the model (1.2) if $\beta_4 > 0$.

The proofs of Lemmas A.2 and A.3 are at the end of the appendix. Let $c = \{z(\alpha)\}^2$.

LEMMA A.1. As $\sigma \to 0$ for $f(0,\beta) > 0$, $\eta_c^* = \sigma a_c + O(\sigma^2)$ and $a_c = (c/M)^{1/2} f^\theta(0,\beta)\{\partial/\partial\eta \; h(0,\beta)\}^{-1}$.

Proof: A Taylor series expansion of (3.1) in η_c^* and around zero.

LEMMA A.2. Assume that as $N \to \infty$, $\sigma \to 0$, $(\hat{\eta}_c - \eta_c^*) = O_p(\sigma N^{1/2})$. Define $A_1 = 2Ma_c\{\partial/\partial\eta\ h(0,\beta)\}^2/\{cf^{2\theta}(0,\beta)\}$. Then as $N \to \infty$, $\sigma \to 0$, if $N^{1/2}(\hat{\theta} - \theta) = O_p(1)$,

$$A_1 N^{1/2}(\hat{\eta}_c - \eta_c^*)/\sigma = N^{1/2}(\hat{\sigma}^2 - \sigma^2)/\sigma^2 + 2\{\log f(0,\beta)\}\ N^{1/2}(\hat{\theta} - \theta) + o_p(1). \qquad (A.5)$$

LEMMA A.3. Consider Lemma A.2. Then

$$(NM)^{1/2}(\hat{\sigma}^2 - \sigma^2)/\sigma^2 = (NM)^{-1/2} \sum_{i=1}^{N} \sum_{j=1}^{M} (\epsilon_{ij}^2 - 1) - 2\bar{v}(NM)^{1/2}(\hat{\theta} - \theta) + o_p(1)$$

so that if $A_0 = M^{1/2}A_1$ and d_0 is defined as in Section 3,

$$A_0 N^{1/2}(\hat{\eta}_c - \eta_c^*)/\sigma = (NM)^{-1/2} \sum_{i=1}^{N} \sum_{j=1}^{M} (\epsilon_{ij}^2 - 1) + 2d_0(NM)^{1/2}(\hat{\theta}-\theta) + o_p(1).$$

PROPOSITION 1 : The results of Theorem 2 hold for $\hat{\eta}_c$, where $\hat{\eta}_c = \hat{\eta}_c(\text{LL})$, $\hat{\eta}_c(\text{MML})$ or $\hat{\eta}_c(\text{PL})$.

Proof of Proposition 1 : From Davidian & Carroll (1987), using Theorem 1 we have that

$$N^{1/2}(\hat{\theta}_{LL} - \theta) = (1/2)\ N^{-1/2} \sum_{i=1}^{N} \{\log q_i^2 - E(\log q_i^2)\}\ (v_i - \bar{v}) + o_p(1) :$$

$$N^{1/2}(\hat{\theta}_{MML} - \theta) = (1/2)\ N^{-1/2} \sum_{i=1}^{N} \{q_i^2 - E(q_i^2)\}\ (v_i - \bar{v}) + o_p(1) :$$

$$N^{1/2}(\hat{\theta}_{PL} - \theta) = (1/2)\ N^{-1/2}\ M^{-1} \sum_{i=1}^{N} \sum_{j=1}^{M} (\epsilon_{ij}^2 - 1)\ (v_i - \bar{v}) + o_p(1) \quad .$$

so that by Lemmas A.1 - A.3 and equation (A.5), we have

$$A_0 N^{1/2}(\hat{\eta}_c(PL) - \eta_c^*)/\sigma$$
$$= (NM)^{-1/2} \sum_{i=1}^{N} \sum_{j=1}^{M} (\epsilon_{ij}^2 - 1)\{1 + d_0(v_i - \bar{v})/\sigma_v^2\} + o_p(1);$$

$$A_0 N^{1/2}(\hat{\eta}_c(LL) - \eta_c^*)/\sigma$$
$$= (NM)^{-1/2} \sum_{i=1}^{N} \sum_{j=1}^{M} (\epsilon_{ij}^2 - 1) + d_0 M^{1/2} N^{-1/2} \sum_{i=1}^{N} (v_i - \bar{v}) \log q_i^2/\sigma_v^2 + o_p(1);$$

and

$$A_0 N^{1/2}(\hat{\eta}_c(MML) - \eta_c^*)/\sigma$$
$$= (NM)^{-1/2} \sum_{i=1}^{N} \sum_{j=1}^{M} (\epsilon_{ij}^2 - 1) + d_0 M^{1/2} N^{-1/2} \sum_{i=1}^{N} (v_i - \bar{v}) q_i^2/\sigma_v^2 + o_p(1).$$

Simple central limit theorem calculations yield the result.

Remark: The result for $\hat{\theta}_{MML}$ is based on $\hat{\sigma}$ obtained from the residuals of the final fit of the mean response function as for the log-linearized method and pseudo-likelihood, so that Lemma A.3 holds. The modified maximum likelihood method also provides a joint estimate of σ along with the estimate of θ. If one considers this estimator in place of $\hat{\sigma}$ in Lemma A.3, it can be shown that the resulting estimator of minimal detectable concentration has even larger asymptotic variance than that in Theorem 2.

Proof of Theorem 2 : By (A.5), for any of the estimators $\hat{x}_c$, since $\eta = \ell(x,\beta)$, we have for some Δ that $\ell(\hat{x}_c,\beta)$ is asymptotically normal with mean $\ell(x_c^*,\beta)$ and variance $\sigma^2\Delta/N$. Thus, for τ_c between $\hat{x}_c$ and x_c^*, defining $W_N = N^{1/2}\ \ell_x(x_c^*,\beta)$ $(\hat{x}_c - x_c^*)\ /\ \sigma$, we have $W_N\ \ell_x(\tau_c,\beta)\ /\ \ell_x(x_c^*,\beta)$ is asymptotically normal with zero mean and variance Δ, where $\ell_x(v,\beta)$ is the derivative of the first component of $\ell(v,\beta)$. It thus suffices through (A.4) to prove that

$\ell(\tau_c,\beta)/\ell(x_c^*,\beta)$ converges in probability to 1. But this follows since $\eta_c^*/\sigma = \ell(x_c^*,\beta)/\sigma \to a_c$, see Lemma A.1. The result now follows from Propostion 1.

Proof of Lemma A.2: By a series of Taylor expansions and using Lemma A.1,

$$N^{1/2}\{h(\hat{\eta}_c,\hat{\beta}) - h(0,\hat{\beta})\}^2/\sigma^2$$
$$= N^{1/2}\{h(\eta_c^*,\beta) - h(0,\beta)\}^2/\sigma^2$$
$$+ 2a_c\{\partial/\partial\eta\ h(0,\beta)\}^2 N^{1/2}(\hat{\eta}_c - \eta_c^*)/\sigma + o_p(1). \qquad (A.6)$$

Similar calculations noting that $N^{1/2}(\hat{\beta} - \beta) = O_p(\sigma)$ and $\eta_c^* \to 0$ yield

$$N^{1/2} h^{2\hat{\theta}}(\hat{\eta}_c,\hat{\beta})\hat{\sigma}^2/(M\sigma^2)$$
$$= \{h^{2\theta}(\eta_c^*,\beta)/M\}N^{1/2} + (c/M)\ h^{2\theta}(0,\beta)N^{1/2}(\hat{\sigma} - \sigma)/\sigma^2$$
$$+ (2c/M)\ h^{2\theta}(0,\beta)\ \{\log h(0,\beta)\}\ N^{1/2}(\hat{\theta} - \theta) + o_p(1). \qquad (A.7)$$

Combining (1.5), (3.1), (A.6) and (A.7) yields (A.5).

Proof of Lemma A.3: Define

$$\hat{\sigma}_0^2 = (NM)^{-1} \sum_{i=1}^{N} \sum_{j=1}^{M} [\{Y_{ij} - f(x_i,\beta)\}/f^{\theta}(x_i,\beta)]^2 = (NM)^{-1}\sigma^2 \sum_{i=1}^{N} \sum_{j=1}^{M} \epsilon_{ij}^2.$$

Then, since $(NM)^{1/2}(\hat{\beta} - \beta) = O_p(\sigma)$,

$$(NM)^{1/2}(\hat{\sigma}^2 - \hat{\sigma}_0^2)/\sigma^2$$
$$= (NM)^{-1/2} \sum_{i=1}^{N} \sum_{j=1}^{M} [\{Y_{ij} - f(x_i,\beta)\}^2/f^{2\hat{\theta}}(x_i,\beta)$$
$$- \{Y_{ij} - f(x_i,\beta)\}^2/f^{2\theta}(x_i,\beta)]\sigma^{-2} + o_p(1)$$

$$= -2\bar{v}(NM)^{1/2}(\hat{\theta} - \theta) + o_p(1),$$

completing the proof.

REFERENCES

Box, G. E. P. (1987). Studies in quality improvement: Signal-to-noise ratios, performance criteria, and statistical analysis. Technometrics, to appear.

Butt, W.R. (1984). Practical Immunoassay. New York: Marcel Dekker, Inc.

Carroll ,R. J. & Ruppert, D. (1982a). A comparison between maximum likelihood and generalized least squares in a heteroscedastic linear model. Journal of the American Statistical Association 77, 878-882.

Carroll, R. J. & Ruppert, D. (1982b). Robust estimation in heteroscedastic linear models, Annals of Statistics 10, 429-441.

Carroll, R. J. & Ruppert, D. (1984). Power transformations when fitting theoretical models to data. Journal of the American Statistical Association 79, 321-328.

Davidian, M. & Carroll, R.J. (1987). Variance function estimation. Journal of the American Statistical Association, to appear.

Finney, D. J. (1964). Statistical Methods in Biological Assay. London: Griffin.

Finney, D. J. (1976). Radioligand assay. Biometrics 32, 721-740.

Finney, D. J. (1978). Statistical Method in Biological Assay, Third Edition. London: Charles Griffin and Company, Ltd.

Giltinan, D. M., Carroll, R. J. & Ruppert, D. (1986). Some new methods for weighted regression when there are possible outliers. Technometrics 28, 219-230.

Jobson, J. D. & Fuller, W. A. (1980). Least squares estimation when the covariance matrix and parameter vector are functionally related. Journal of the American Statistical Association 75, 176-181.

McCullagh, P. (1983). Quasi-likelihood functions. Annals of Statistics 11, 59-67.

Oppenheimer, L., Capizzi, T.P., Weppelman, R.M. & Mehto, H. (1983). Determining the lowest limit of reliable assay measurement.

Analytical Chemistry 55, 638-643.

Raab, G. M. (1981a). Estimation of a variance function, with application to radioimmunoassay. Applied Statistics 30, 32-40.

Raab, G. M. (1981b). Letter on 'Robust calibration and radioimmunoassay.' Biometrics 37, 839-841.

Rodbard, D. (1978). Statistical estimation of the minimum detectable conentration ("Sensitivity") for radioligand assays. Analytical Biochemistry 90, 1-12.

Rodbard, D. & Frazier, G.R. (1975). Statistical analysis of radioligand assay data. Methods of Enzymology 37, 3-22.

Sadler, W.A. & Smith, M.H. (1985). Estimation of the response-error relationship in immunoassay. Clinical Chemistry 31/11, 1802-1805.

Stefanski, L. A. & Carroll, R.J. (1985). Covariate measurement error in logistic regression. Annals of Statistics 13, 1335-1351.

Tiede, J.J. & Pagano, M. (1979). The application of robust calibration to radioimmunoassay. Biometrics 35,567-574.

Wolter, K. M., & Fuller, W. A. (1982). Estimation of nonlinear errors-in-variables models. Annals of Statistics 10, 539-548.

Table 1. Data for example of Section 5

Concentration (x)	Response (Y)
0.000	1.700, 1.660, 1.950, 2.070
0.075	1.910, 2.270, 2.110, 2.390
0.1025	2.220, 2.250, 3.260, 2.920
0.135	2.800, 2.940, 2.380, 2.700
0.185	2.780, 2.640, 2.710, 2.850
0.250	3.540, 2.860, 3.150, 3.320
0.400	3.910, 3.830, 4.880, 4.210
0.550	4.540, 4.470, 4.790, 5.680
0.750	6.060, 5.070, 5.000, 5.980
1.000	5.840, 5.790, 6.100, 7.810
1.375	7.310, 7.080, 7.060, 6.870
1.850	9.880, 10.120, 9.220, 9.960
2.500	11.040, 10.460, 10.880, 11.650
3.250	13 10, 15.470, 14.210, 13.920
4.500	16.070, 14.670, 14.780, 15.210
6.000	17.340, 16.850, 16.740, 16.870
8.250	18.980, 19.850, 18.750, 18.510
11.250	21.666, 21.218, 19.790, 22.669
15.000	23.206, 22.239, 22.436, 22.597
20.250	23.922, 24.871, 23.815, 24.871
27.500	25.748, 25.874, 24.907, 24.871
37.000	24.441, 25.874, 25.748, 27.270
50.000	29.580, 26.698, 26.536, 27.181

Table 2. Estimates of θ and x_c based on example of Section 5

	$\hat{\theta}_{PL}$	$\hat{\theta}_{LL}$	$\hat{\theta}_{MML}$	$\hat{x}_c$(LS)	$\hat{x}_c$(PL)	$\hat{x}_c$(LL)	$\hat{x}_c$(MML)
Full Duplicates	0.4750	0.4757	0.4500	0.1554	0.0790	0.0793	0.0822
1 & 2	0.7000	0.9404	0.7500	0.2230	0.0728	0.0476	0.0659
2 & 3	0.3500	0.1950	0.2500	0.2385	0.1555	0.1870	0.1739
3 & 4	0.5750	0.6940	0.7000	0.2513	0.1324	0.1112	0.1104
1 & 4	0.5500	0.5931	0.5000	0.1593	0.0612	0.0601	0.0695
1 & 3	0.4500	0.4233	0.4750	0.1859	0.0938	0.0981	0.0909
Mean	0.5250	0.5692	0.5350	0.2116	0.1031	0.1008	0.1021
SD	0.1183	0.2511	0.1997	0.0763	0.0357	0.0491	0.0439

Note: Means and SDs are based only on the five reduced combinations of the data with duplicates.

Estimate of minimum detectable concentration based on unweighted least squares is denoted $\hat{x}_c$(LS).